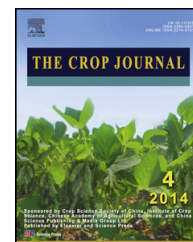


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Effect of different levels of nitrogen deficiency on switchgrass seedling growth

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ABSTRACT

Switchgrass (*Panicum virgatum* L.) is a warm-season rhizomatous perennial grass that can tolerate diverse abiotic stresses while yielding relatively high biomass, and is considered a leading biofuel feedstock for marginal lands. Nitrogen (N) is crucial for the growth and development of switchgrass, and its tolerance to low N supply and high N use efficiency are very important for its production under poor conditions. The large-scale planting of switchgrass on marginal lands could be an effective approach to solving the problem of feedstock supply for biomass energy. This study used a hydroponic experiment to evaluate the effect of N deficiency on switchgrass seedlings. Three N treatments (0, 0.15, and 1.50 mmol L⁻¹ Hoagland's solution) and six cultivars were used, three of each ecotype (upland and lowland). The results showed that biomass, leaf area, root surface area, net photosynthesis, and total chlorophyll content significantly decreased under low N treatments compared with those in full strength Hoagland's nutrient solution. However, once established, all plants survived extreme N stress (0 mmol L⁻¹) and, to some extent, were productive. Cultivar Kanlow performed best of the six cultivars under stress. Significant interactions between stress treatment and cultivars showed that breeding for cultivars with high yield and superior performance under N deficiency is warranted. The lowland outperformed the upland ecotypes under stress, suggesting that lowland cultivars may survive and be productive under a wider range of stress conditions. However, given the better adaptability of lowland ecotypes to hydroponic cultivation, further study is needed.

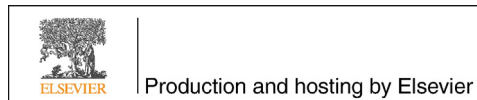
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1. Introduction

Increasing energy security and mitigating climate change are the two main motives that have pushed renewable energy production to the top of global agendas [1]. They are encouraging the agronomic production of biomass to help meet renewable bioenergy needs. Perennial grasses are attractive as biomass sources, as they can meet the agronomic, environmental and social requirements for successful deployment as energy crops. Perennial rhizomatous grass is an ideal biofuel crop, because it displays the agronomically desirable traits of broad climatic tolerance, rapid growth rates, and relatively high yield. Furthermore, owing to the recycling of nutrients by their rhizome systems, perennial grasses have a low nutrient demand [2]. They are also seldom attacked by pests and so can be produced with few or no pesticides [3].

Given these unique advantages, the interest in using biofuel crops for energy production is soaring. However, because China cannot afford biomass energy production from its croplands [4], biofuel cultivation, to be competitive with conventional energy sources and avoid the supplantation of food crops, will likely be relegated to less productive soils and will receive minimal inputs of water, fertilizer, and pesticides [5]. Thus, marginal lands may play an important role in biomass energy production. It is estimated that the quantity of marginal land that could be used in biofuel production in China is near 110 million ha, of which about 45 million ha would support economic operation [4]. Abiotic stresses including lack of nutrients, drought, and high salt levels in these areas are common factors that will limit the production of biofuel crops.

Under environmental stress such as nitrogen (N) deficiency, which will be a major limiting factor to cultivating biofuel crops in northwestern and northern China, plants show varying adaptations at the morphological, biochemical, molecular and physiological levels. It is imperative to increase our knowledge on the tolerance of biofuel crops to diverse nutrient deficiency conditions to allow continuous biomass industrialization on marginal lands. Efficient production of bioenergy from such marginal lands requires the choice of the most stress-tolerant grass species. Biofuel crops are being screened for superior characteristics or bred and genetically modified for enhanced abiotic stress tolerance traits that will expand their cultivable area [6]. It is accordingly desirable to evaluate the responses of promising biofuel crops to N-deficiency stress and identify cultivars that are most suitable for biomass production under N-deficiency conditions.

Switchgrass (*Panicum virgatum* L.) is a warm-season rhizomatous perennial C₄ grass that originated in the North American tall grass prairie. It is a leading dedicated biofuel feedstock candidate in the United States, owing to its broad adaptability, rapid growth rate, ability to grow in low productivity soils, and ability to function as one component in a multipurpose cropping system [7,8]. It responds strongly to N fertilizer and is often drought tolerant [9–12]. It can effectively sequester carbon in the soil, and provide excellent cover for wildlife [13,14]. With many beneficial attributes as energy crops, the Department of Energy's Bioenergy Feedstock Development Program (BFDP) decided to focus research on a model crop system and to concentrate

research resources on switchgrass, in order to rapidly realize its maximal output as a biomass crop [15].

There are two distinct ecotypes of switchgrass: lowland tetraploid and upland octoploid. The lowland tetraploid ecotype originates primarily in the southern extent of the native range and the upland octoploid primarily in its middle to northern extent [7]. Several dozen cultivated varieties of each ecotype are commercially available, most of which are high-yielding selections from native populations [7]. The species shows wide variation in performance relative to environmental variables, though lowland ecotypes typically produce larger yields than upland ecotypes [16].

Previous studies have focused mainly on the responses of switchgrass biomass to N nutrient application [17–19]. The effect of N deficiency on switchgrass has not been extensively studied, especially for hydroponically cultivated seedlings, and knowledge of the effects of various levels of N deficiency on agronomic traits, photosynthetic parameters, and chlorophyll content in switchgrass is limited. The objective of this study was to evaluate the performance and reproductive potential of six cultivars from the two ecotypes in response to N deficiency stress and provide some theoretical basis for relatively high-yield cultivation of switchgrass in low-fertility soils and for breeding for high N use efficiency.

2. Materials and methods

2.1. Materials

Six cultivars of two switchgrass ecotypes, including the lowland ecotypes Alamo, Kanlow, and BJ-1 and the upland ecotypes Forestburg, Pathfinder, and Trailblazer were used (Table 1). Seeds were obtained from the National Demonstration for Precision Agriculture Experiment Station (39°34' N, 116°28' E) in Changping District, Beijing, China.

2.2. Experimental design

The experiment was performed in a greenhouse at the Beijing Academy of Agriculture and Forestry Sciences. Conditions were a 29/21(±2) °C day/night cycle with 32.2%–53.0% humidity. Sodium lamps were used to maintain a 12-hour photoperiod with an illumination intensity of 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Each treatment had eight replications laid out in a completely randomized design. Seeds of each cultivar were disinfected in 9% hydrogen peroxide solution for 30 min, rinsed three times with distilled water, and sown in flats filled with washed sand on July 20th 2010. Five weeks after germination, uniform seedlings with two leaves were selected and transplanted into 14 L plastic pots (41.0 cm × 30.5 cm × 13.5 cm) containing full-strength Hoagland's nutrient solution, modified in a random complete block design for eight replications [20]. Seedlings of each cultivar were then exposed to different N deficiency stress treatments at the five-leaf stage.

Hoagland's solution without N [$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$] was then added to maintain various N deficiency treatments [20], including mild stress [N2: $1.5 \text{ mmol L}^{-1} \text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$], moderate stress (N1: 0.15 mmol L^{-1}), extreme stress (N0: 0 mmol L^{-1}) and a stress-free control (full strength Hoagland's nutrient solution, modified). The solutions were refreshed twice a week and the pH of the nutrient solutions was adjusted to 5.5–6.5 every 2 days. An air pump was used for ventilation 24 h per day. Agronomic and physiological traits were evaluated 60 days after treatment.

2.3. Data collection

Sixty days after treatments, the tiller number, height (from the pot surface to the end of the longest leaf on the tallest tiller), aboveground biomass, leaf area, and root area were measured. Aboveground biomass was cut at the pot surface and separated into shoots and leaves, the leaf area was determined with a LI-COR 3100 leaf area meter (Li-Cor, Lincoln, NE) and the root surface area was determined with a root scanner (Epson Expression 1000XL, Japan). Roots and rhizomes were washed free of growth media and all plant samples were treated at 105°C for 30 min for fixation and then oven dried at 65°C until a constant weight was reached. The presence of rhizomes was recorded and the root to shoot weight ratio (R:S) was calculated.

Gas exchange measurements were performed two weeks after treatment initiation using a portable open gas exchange system (LI-6400, LI-COR) calibrated to deliver a photosynthetic photon flux density of $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ and an ambient CO_2 of $400 \mu\text{mol mol}^{-1}$ (supplied by a LI-COR CO_2 injector) and a leaf temperature of $(30 \pm 1)^\circ\text{C}$. Data were collected for 2 min at 5-s intervals for three randomly chosen plants from each treatment listed above (eight replications per treatment) on the youngest fully expanded leaf on the longest tiller, as described by Barney et al. [12]. Net CO_2 assimilation (A), transpiration (E), and stomatal conductance (g_s) were recorded, and photosynthetic water use efficiency (WUE) was calculated ($\text{WUE} = \text{net photosynthesis/transpiration}$).

Chlorophyll a and chlorophyll b were extracted with 80% acetone from the same leaf as used for gas exchange measurements. Absorbance was measured at 663 nm and 645 nm for chlorophyll a and chlorophyll b, respectively, using a UV spectrophotometer (UV-2550, Shimadzu). Total chlorophyll content was calculated according to the procedure described by Lichtenthaler and Wellburn [21].

To avoid the negative influence of different cultivars on the evaluation of tolerance, the Low-N tolerance index (LNT) was calculated. This is the ratio of the index under treatment to that of the control ($\text{LNT} = (\text{value of tested traits under treatments} / \text{value of same tested traits under control}) \times 100\%$).

2.4. Data analysis

All data were subjected to analysis of variance (ANOVA) with ecotype, cultivar nested within ecotype, N deficiency treatment, and ecotype-by-treatment and cultivar-by-treatment interactions as fixed effects. All analyses were performed with SAS V8.2 statistics software. All means and standard errors are presented as untransformed values.

3. Results

3.1. Effect of nitrogen deficiency on agronomic traits of switchgrass seedlings

Besides tiller number, all other agronomic traits differed across cultivars (Table S1), with Kanlow displaying more biomass, leaf area and root surface area, and longer culms across all N deficiency treatments (Fig. 1). No significant difference was observed between Alamo and Kanlow in any traits but tiller number (Fig. 1). All cultivars of lowland ecotypes outperformed upland cultivars, and no significant difference was observed among upland cultivars for any trait (Fig. 2). There were significant cultivar-by-treatment and ecotype-by-treatment interactions for all agronomic traits except tiller number. Tiller number showed only extremely strong responses to treatment (Table S1).

Aside from tiller number and R:S, all other agronomic traits varied across ecotypes (Table S1), with lowland cultivars producing 47% more biomass, 58% longer culms, 48% more leaf area, and 42% more root surface area than upland cultivars (Fig. 2).

Nitrogen deficiency affected agronomic traits, and all traits showed large differences across the four treatments, with the control yielding an average of 168% more total biomass, 148% more aboveground biomass, 189% more belowground biomass, 53% more tillers, 127% more leaf area, 99% more root surface area, and 58% longer culms than the N deficiency treatments (Table 2). Clearly, cultivars performed best under the control conditions, followed by moderate stress, and worst under extreme stress. No significance for R:S was observed between the control and N1 or N2. Tiller number, leaf area, root surface area, total biomass, aboveground biomass, and belowground biomass under the N2 treatment were significantly higher than under the N1. Height and belowground biomass did not differ between the N1 and N0 treatments (Table 2).

Surprisingly, there were highly significant interactions between stress treatments and cultivars for all agronomic traits but tiller number (Table S1); response to N deficiency stress depended on cultivar.

For Alamo, height showed no difference across the three stress levels (Fig. 3-A); for Pathfinder, height and aboveground biomass did not differ between the N1 and N2 treatments (Fig. 3-A, D). For both ecotypes, all the agronomic traits varied across N stress treatments (Fig. 3).

According to Fig. 3, accumulation can also be calculated in height, leaf area, root surface area, aboveground biomass, belowground biomass and total biomass with decreasing N level for each cultivar (data not shown). Kanlow had the lowest overall response to decreasing N concentration for the agronomic traits in Fig. 3, immediately followed by Alamo. Kanlow also showed the best performance under the three N stress treatments for all the traits. For height, leaf area and root surface area, Pathfinder had the overall highest effect of decreased N level (Fig. 3-A, B, C), the highest response for height under N2 and N0 treatments (Fig. 3-A), the highest response for leaf area under N2, N1, and N0 treatments (Fig. 3-B), and the highest response for root surface area under the N1 and N0 treatments (Fig. 3-C). For aboveground biomass,

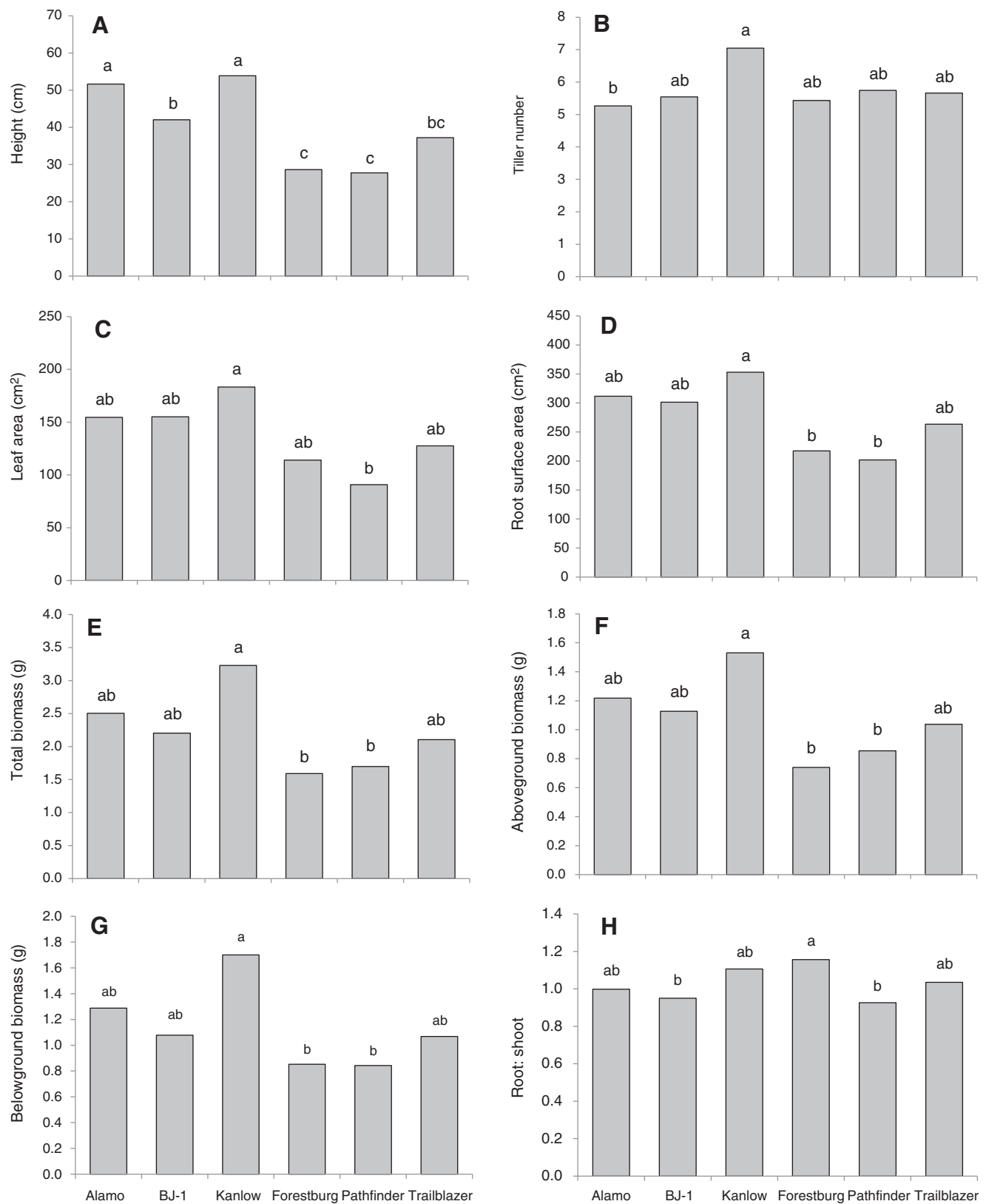


Fig. 1 – Cultivar means over all conditions for (A) height, (B) tiller number, (C) leaf area, (D) root surface area, (E) total biomass, (F) aboveground biomass, (G) belowground biomass, and (H) R:S. Cultivars with different letters are significantly different at $P < 0.05$.

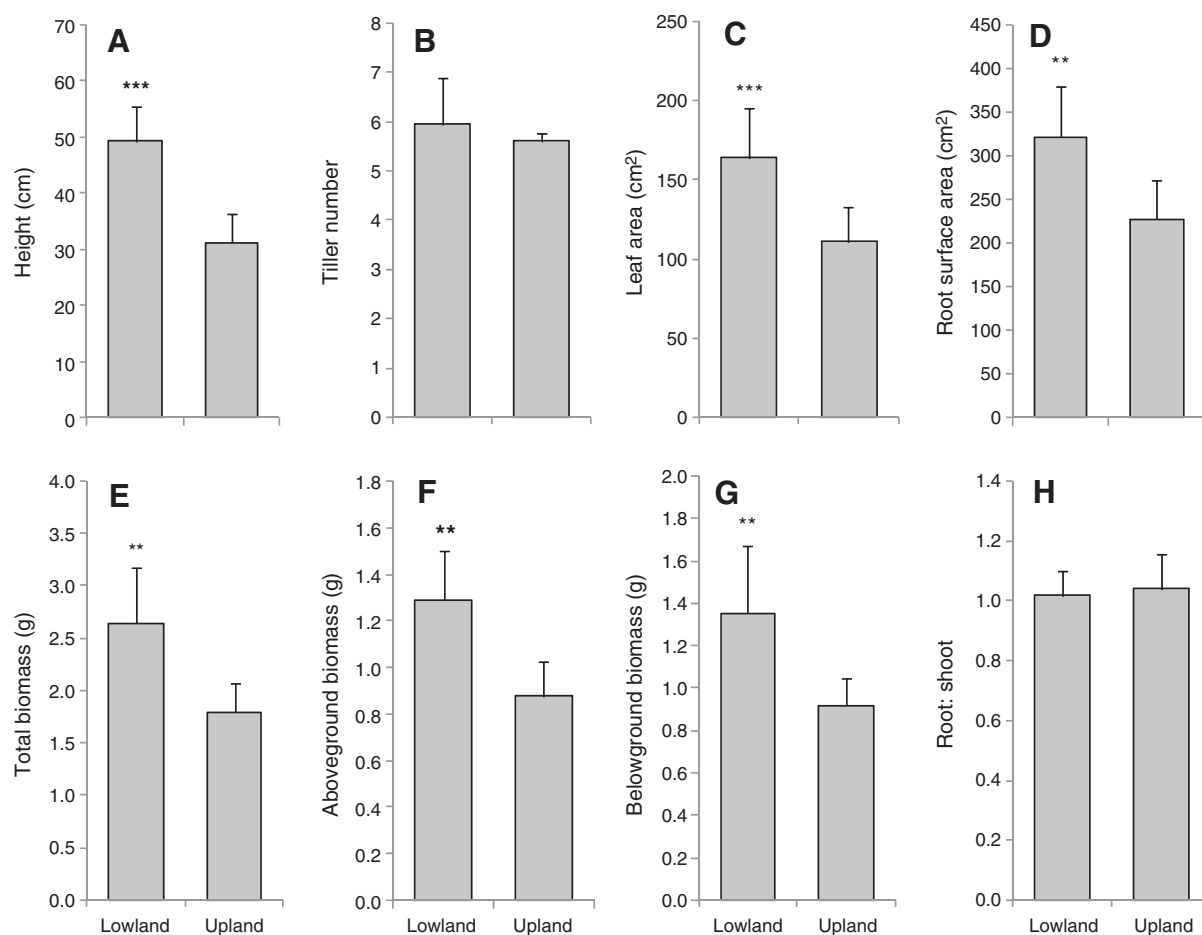


Fig. 2 – Ecotype means over all conditions for (A) height, (B) tiller number, (C) leaf area, (D) root surface area, (E) total biomass, (F) aboveground biomass, (G) belowground biomass, and (H) R:S. Asterisks represent ecotypic differences (* $P < 0.05$, ** $P < 0.01$, * $P < 0.001$).**

Forestburg had the highest overall response to decreasing N concentration and the worst performance under all treatments (Fig. 3-D). For belowground, Trailblazer had the highest overall response to decreasing N concentration (Fig. 3-E, F), but only with the highest response under N0 treatment for belowground biomass (Fig. 3-E).

Lowland ecotypes had a lower response than upland ecotypes to decreasing N concentration (Fig. 4). The cultivars responded differently for most agronomic traits when the N deficiency stress was varied.

3.2. Effect of different nitrogen deficiency levels on physiological traits of switchgrass seedlings

All physiological traits were affected by N deficiency stresses. Only chlorophyll content differed among cultivars (Table S2), with that of Kanlow 1.4% higher than that of all other cultivars (data not shown). A and E were 31% and 23% higher, respectively, in lowland than in upland ecotypes, but there was no significant difference in these two traits observed across cultivars (Table S2, Figs. 5 and 6). The N deficiency treatments affected the photosynthetic indices and there was a decrease in A, E, and g_s compared with the control. A similar trend was found with chlorophyll content. All traits showed extreme differences across the four treatments and cultivar-by-treatment interaction. There was no significant ecotype-by-treatment interaction in WUE and chlorophyll content (Table S2).

Notably, cultivars performed best under the control condition, followed by moderate stress, and worst under extreme stress (Table 3), suggesting that switchgrass suffered reduced A by an average of 43%, E by 32%, g_s by 34%, WUE by 19%, and chlorophyll content by 46% compared with the control (Table 3).

Table 1 – Cultivars used in this experiment.

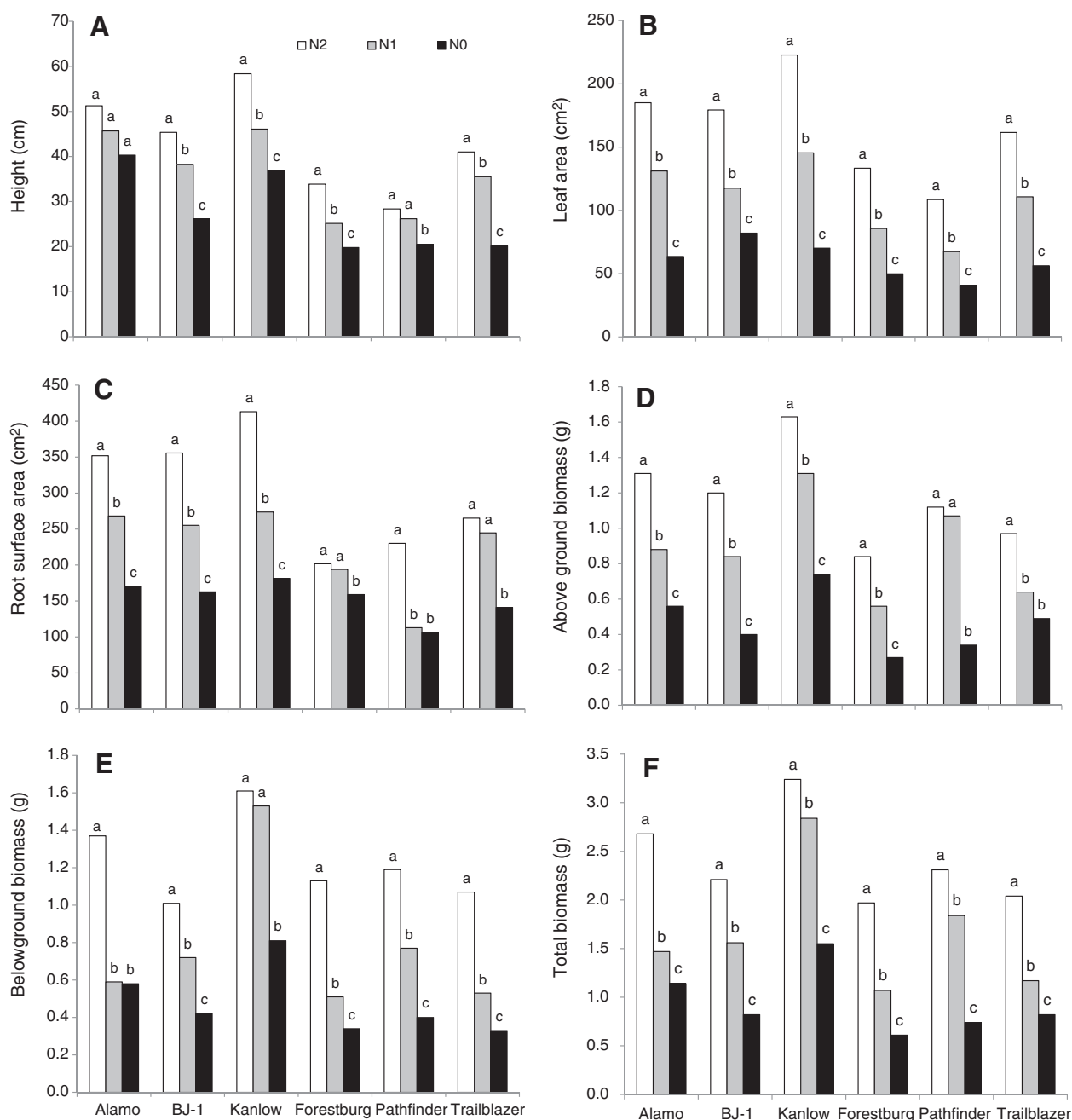
Cultivar	Ecotype	Ploidy	Origin
Alamo	Lowland	Tetraploid	South Texas, U.S.A.
BJ-1 ^a	Lowland	–	–
Kanlow	Lowland	Tetraploid	Central Oklahoma, U.S.A.
Forestburg	Upland	Tetraploid	South Dakota, U.S.A.
Pathfinder	Upland	Octoploid	Nebraska/Kansas, U.S.A.
Trailblazer	Upland	Octoploid	Nebraska, U.S.A.

^a The ploidy and origin of BJ-1 are unknown.

Table 2 – Effects of various N deficiency stress levels on eight agronomic traits of switchgrass.

Treatment	Height (cm)	Tiller number per plant	Leaf area (cm ²)	Root surface area (cm ²)	Total biomass (g)	Aboveground biomass (g)	Belowground biomass (g)	R:S
CK	54.28 a	7.62 a	214.79 a	417.94 a	3.88 a	1.81 a	2.07 a	1.14 a
N2	43.04 b	6.46 b	165.14 b	302.98 b	2.41 b	1.18 b	1.23 b	1.06 a
N1	36.15 bc	5.02 c	109.66 c	224.74 c	1.65 c	0.88 c	0.77 c	1.05 a
N0	27.29 c	4.03 d	60.47 d	153.53 d	0.95 d	0.47 d	0.48 c	0.86 b

Different letters indicate significant differences in an agronomic trait between the four treatments ($P < 0.01$).

**Fig. 3 – Cultivar means under N1, N2, and N0 conditions for (A) height, (B) leaf area, (C) root surface area, (D) aboveground biomass, (E) belowground biomass, and (F) total biomass. Cultivars with different letters are significantly different at $P < 0.05$.**

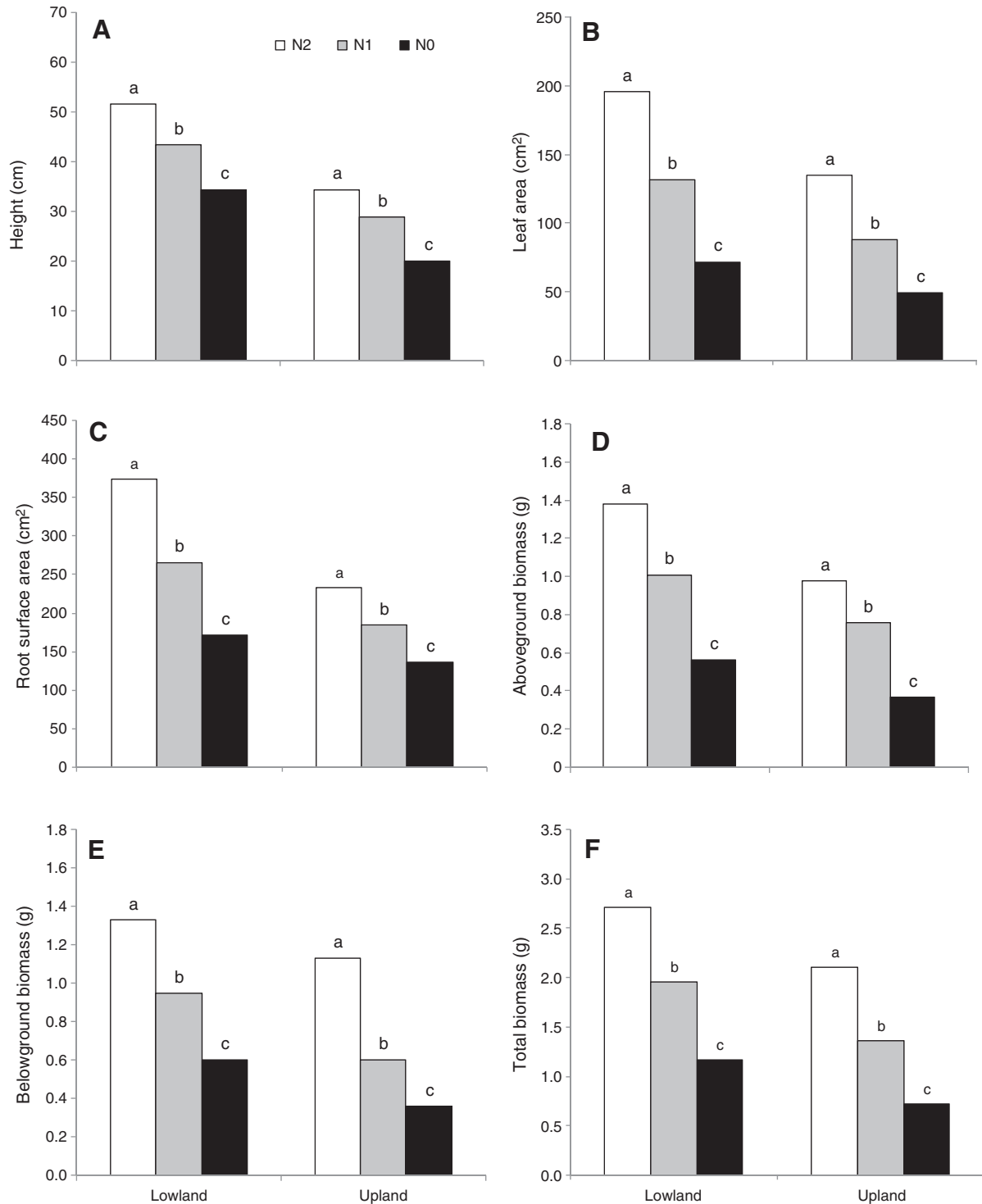


Fig. 4 – Ecotype means under N1, N2, and N0 conditions for (A) height, (B) leaf area, (C) root surface area, (D) aboveground biomass, (E) belowground biomass, and (F) total biomass. Ecotypes with different letters are significantly different at $P < 0.05$.

There were highly significant cultivar-by-treatment interactions for all physiological traits (Table S2), meaning that the response to N deficiency stress depended on cultivar.

For the six cultivars, A, E, g_s , and chlorophyll content all showed differences across the N2, N1, and N0 treatments (Fig. 7). For both ecotypes, all of the physiological traits varied across N stress treatments (Fig. 8).

According to Fig. 7, accumulation can also be calculated in A, E, g_s , and chlorophyll content with increasing stress level for each cultivar (data not shown). For A and E, Kanlow had the lowest overall response and performed best under N2 and N1 treatments, while Pathfinder had the highest overall response to decreasing N level, especially under mild stress (Fig. 7-A, B). For g_s , Trailblazer had the lowest overall response

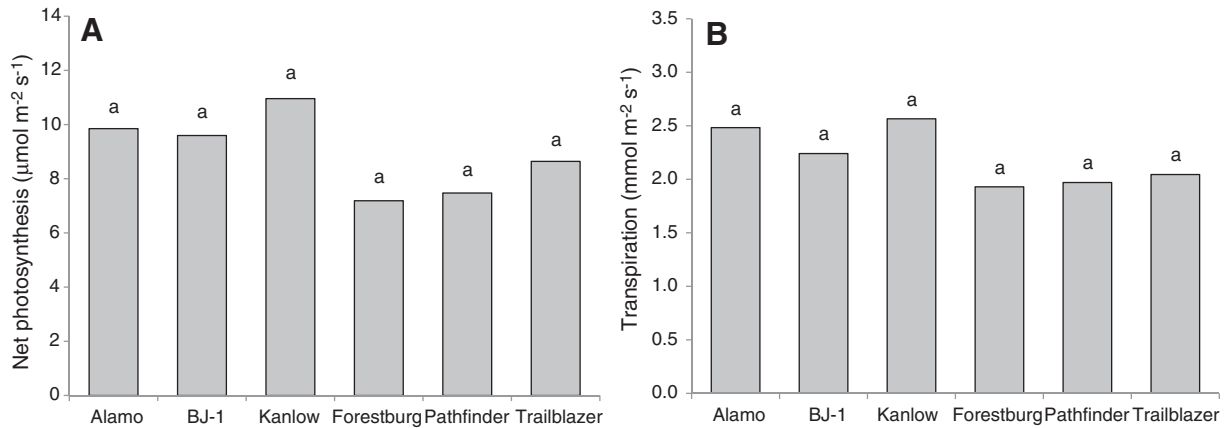


Fig. 5 – Cultivar means over all conditions for (A) net photosynthesis and (B) transpiration. Cultivars with different letters are significantly different at $P < 0.05$.

to decreasing N concentration and performed best under N1 and N0 treatments, while Pathfinder had the highest overall response, especially under N1 and N0 treatments (Fig. 7-C). Surprisingly, for chlorophyll content, Pathfinder had the lowest overall response to increasing stress and performed best under N0 treatment, while Forestburg had the highest overall response, especially under the N2 and N1 treatments (Fig. 7-D).

For the four physiological traits, the accumulation of the lowland was higher than that of the upland ecotypes with increasing stress (Fig. 8). Obviously, the cultivars respond differently with respect to physiological traits when N deficiency stress is altered.

3.3. Evaluation indices for the performance of switchgrass seedlings under different nitrogen deficiency levels

The LNT of all of the screened evaluation indices showed highly significant differences across three treatments (Table 4). For N2,

total biomass and height, followed by A, suffered the greatest reduction compared with other indices. For N1, height and A showed higher performance than other indices and total biomass and leaf area declined the most compared with other indices. Total biomass was the most sensitive index under the three N deficiency treatments and height was the most insensitive index across all stress levels.

4. Discussion

Among the abiotic variables regulating the habitat suitability for a species, N availability is crucial. Nitrogen is one of the most important nutrients for crop growth and development because it affects dry matter production by influencing the leaf area development and maintenance as well as photosynthetic efficiency. In addition, N deficiency reduces radiation interception, radiation use efficiency, dry matter partitioning to reproductive organs, leaf area index, and the protein content of the plant and seed [22]. The detailed effects of N deficiency on crop yield depend on the growth stage at which it occurs, as well as on its duration and extent [23]. In this experiment, biomass, leaf area, root surface area, tiller number and height showed considerable decreases at varying N deficiency levels, in comparison to standard N supply. Rates of net photosynthesis and transpiration, stomatal conductance, and chlorophyll content were severely restricted by N deprivation, indicating that primary metabolism was severely limited by low or no N availability.

The net photosynthesis rate of switchgrass decreased under N deficiency treatments as observed in other studies [24]. This effect is attributed mainly to the deficient supply of N for chloroplast protein synthesis. Under low N levels, lower photosynthesis is often attributed to reduction in chlorophyll content and Rubisco activity [25,26]. Also, because N is used by plants to synthesize amino acids and nucleic acids that are necessary for all functions of the plant, a deficiency of N would result in a reduction of net photosynthesis rate.

The WUE indicates the performance of a crop that is grown under any environmental constraint [27]. Application of N

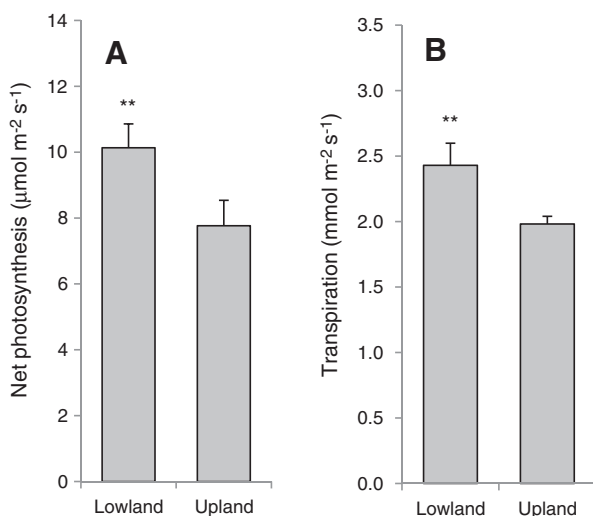


Fig. 6 – Ecotype means over all conditions for (A) net photosynthesis and (B) transpiration. Asterisks represent ecotypic differences ($P < 0.01$).

Table 3 – Effects of various N deficiency stress levels on five physiological traits of switchgrass.

Treatment	Net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$)	Transpiration ($\text{mmol m}^{-2} \text{s}^{-1}$)	WUE ($\mu\text{mol mmol}^{-1}$)	Chlorophyll content ($\text{mg g}^{-1} \text{FW}$)
CK	13.35 a	209.04 a	2.92 a	4.56 a	3.81 a
N2	10.69 b	166.49 b	2.52 b	4.23 a	2.89 b
N1	7.89 c	137.22 c	2.09 c	3.78 b	2.16 c
N0	3.88 d	105.87 d	1.29 d	3.09 c	1.16 d

Different letters indicate significant differences between treatments in each physiological trait ($P < 0.01$).

influences both the amount of water extracted by a crop and crop growth, and consequently can affect WUE. Optimal N levels increase the root surface area and depth as well as root biomass and thus alleviate drought effects. However, the effects of N on crop water use are expected to vary with culture conditions. Under hydroponic conditions with diverse N deficiency levels, the root surface area and belowground biomass of switchgrass were reduced by deficient N (Table 2), so that WUE decreased as N decreased (Table 3).

The rate of transpiration is directly related to the degree of stomatal opening, and to the evaporative demand of the atmosphere surrounding the leaf. Deficiency of N can influence stomatal opening, and thus transpiration rate. There are contradictory conclusions in the literature about the influence of N deficiency on stomatal conductance. Lower rates of stomatal conductance in low-N-grown plants have been reported [28,29], but the opposite or no effect of N application is also reported [26,30]. Possible reasons could lie in the choice of tested materials and experimental conditions. In the present study, under N deficiency stress, the stomatal conductance of switchgrass decreased considerably (Table 3). Given that the amount of transpiration by a plant depends on the number and size of leaves, leaf areas, and plant roots, seedlings grown with nutrient solution lacking N showed a drop in transpiration rate (Table 3). Full-strength Hoagland's nutrient solution treatment supported the highest value of transpiration because of the increased photosynthesis and stomata conduction. There is a linear correlation between photosynthesis and transpiration [31,32].

Thus, for hydroponically cultivated switchgrass, deficient N supply affected the chlorophyll content and stomatal opening and thereby the leaf area and photosynthetic characteristics. This effect reduced the plant's ability to manufacture carbohydrates by photosynthesis and consequently reduced its biomass. The results agree with the findings by Stroup et al. and Kering et al. [24,33].

All the traits showed obvious differences among the applied N deficiency stresses (Tables 2 and 3), suggesting that switchgrass responds strongly to N. However, the tiller number showed no significant difference across cultivars and ecotypes and no cultivar-by-treatment and ecotype-by-treatment interactions (Table S1). One possible explanation would be that the six chosen switchgrass cultivars simply show no difference in tiller number. This could also explain why R:S showed no difference across ecotypes but showed highly significant differences across treatments.

There is no current index for evaluating the tolerance of switchgrass to mineral nutrient deficiency conditions.

According to previous indoor and field study experiments, combined with the physiological characteristics of switchgrass, total biomass, height, tiller number, leaf area, root surface area, net photosynthesis and chlorophyll content were chosen as evaluation indices for effectively measuring its performance. These data showed that although the six cultivars of switchgrass showed significantly reduced performance, they could survive and might be productive (particularly Kanlow), in ecosystems with varying levels of N deficiency. There were also obvious differences among the cultivars in agronomic traits (Fig. 1). Kanlow outperformed Alamo, although for most of the agronomic and physiological characteristics there was no difference between the two cultivars (Fig. 1), a result that disagrees with other studies [24]. A possible reason for this discrepancy is the use of different rates of N and the use of hydroponic instead of field conditions. Kanlow would undoubtedly be the best candidate for cultivation on marginal land with N deficiency. With improvement of infertile lands, cultivation of the Alamo cultivar might also be possible. Lowland outperformed upland ecotypes under N deficiency stress conditions for the agronomic and physiological traits, as was found in another study [24]. Biomass, leaf area, root surface area, height, net photosynthesis, and chlorophyll content were 47%, 48%, 42%, 58%, 30%, and 21% higher, respectively, in lowland than upland ecotypes (Table S1 and Fig. 2). Strong physiological and agronomic responses to the cultivar-by-treatment interaction were also noticed, indicating that for maximum production and optimal performance under multiple N deficiency stresses, proper plantation management (such as choice of cultivars) is required for switchgrass. Based on this experiment, lowland ecotypes can survive under broad N deficiency conditions and may be productive under a wider range of stress conditions, and should be candidates for future genetic and agronomic improvement. However, given the better adaptability of lowland ecotypes to hydroponic conditions, further study is needed.

Switchgrass displays broad tolerance to N deficiency stresses by surviving and yielding under stress. The results likely represent a test of two suitable ecotypes over a range of conditions. The information presented here will aid biomass producers in making crop selection decisions. Environmental variation throughout its vast native range has likely led to this adaptive tolerance, which appears greater in current cultivars than in previously tested wildtypes [34]. The present experiments do not directly address competition in field environments, which will influence both the ability of the crop to establish in minimally managed environments regardless of N deficiency stress tolerance, and the economics of production.

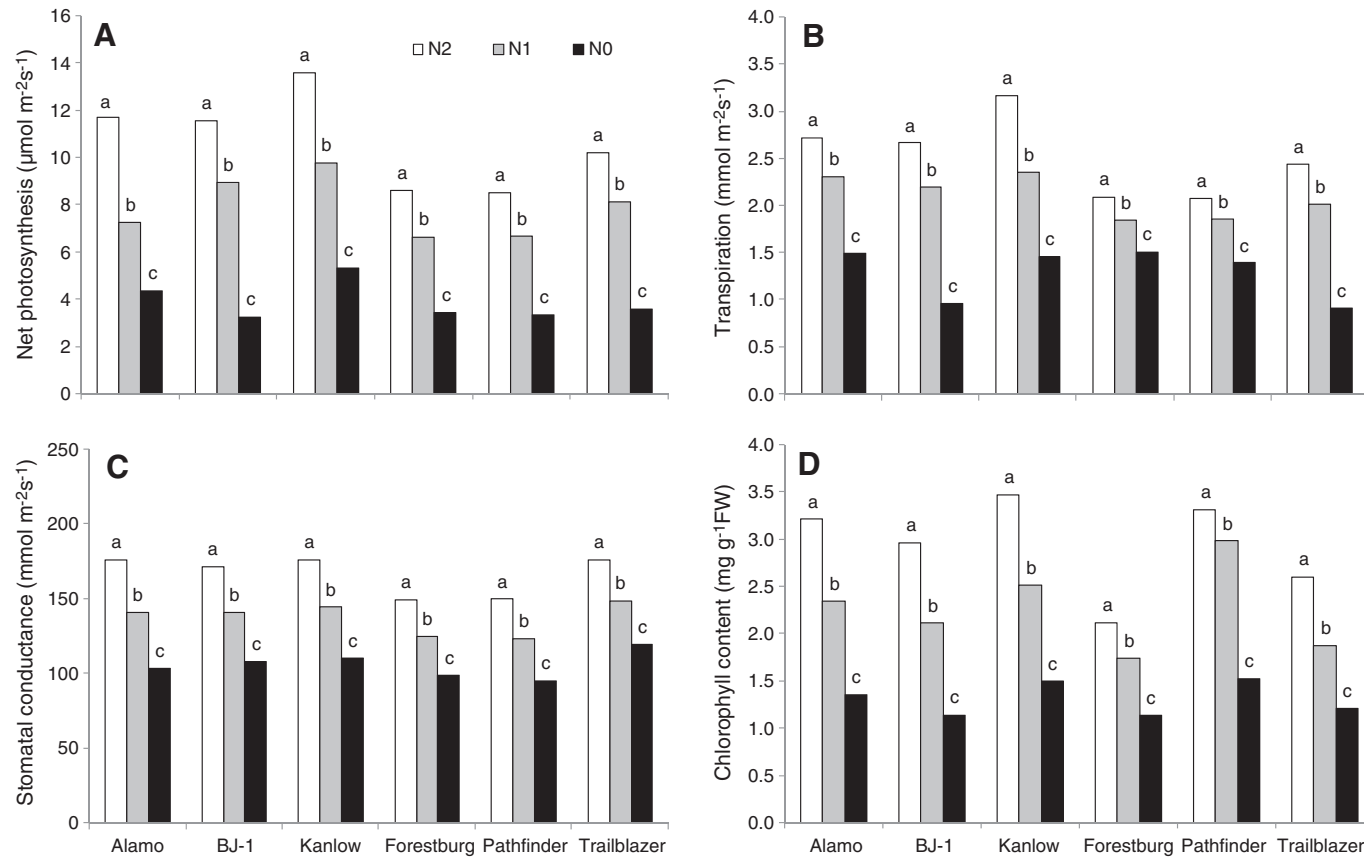


Fig. 7 – Cultivar means under N1, N2, and N0 conditions of (A) net photosynthesis, (B) transpiration, (C) stomatal conductance, and (D) chlorophyll content. Cultivars with different letters are significantly different at $P < 0.05$.

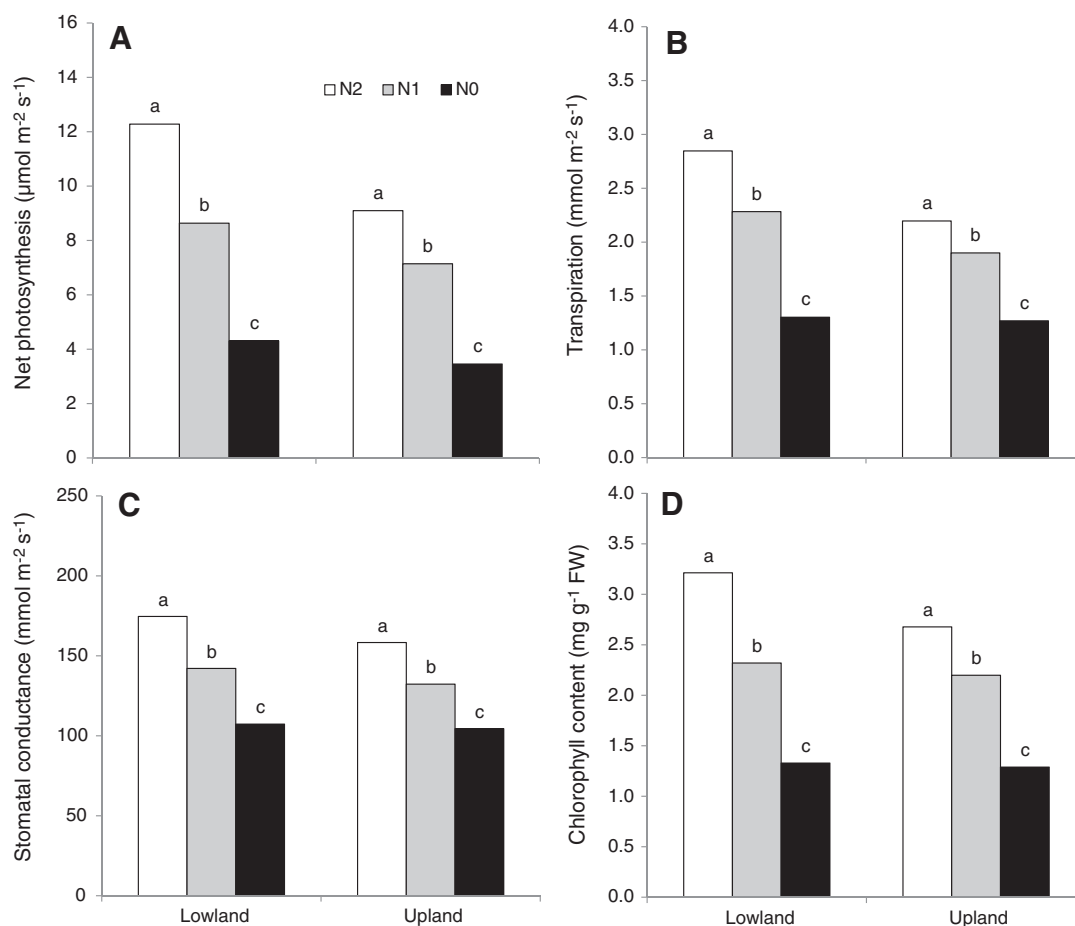


Fig. 8 – Ecotype means under N1, N2, and N0 conditions of (A) net photosynthesis, (B) transpiration, (C) stomatal conductance, and (D) chlorophyll content. Ecotypes with different letters are significantly different at $P < 0.05$.

Equal attention should be paid to this point, as it also plays a vital role in determining the feasibility of switchgrass in marginal lands for biofuel purposes. More studies are necessary to evaluate tolerance to other environmental variables and their interactions with competitive ability.

Acknowledgments

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Supplementary material

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.cj.2014.04.005>.

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Table 4 – Low-N tolerant indexes of different evaluation indices in N deficiency treatment.

Treatment	Total biomass	Height	Leaf area	Root surface area	Net photosynthesis	Chlorophyll content
N2	0.64 a	0.81 a	0.77 a	0.72 a	0.80 a	0.76 a
N1	0.43 b	0.68 b	0.51 b	0.54 b	0.60 b	0.56 b
N0	0.24 c	0.51 c	0.28 c	0.37 c	0.29 c	0.31 c

Different letters indicate significant differences between treatments within columns ($P < 0.01$).

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